

WHAT IS CLAIMED IS:

- 09975650-101.101
1. A method for measuring ligand binding to a vanilloid receptor comprising the steps of:
 - 5 (a) forming in an aqueous solution having a pH in the range of about 7.5 to about 10.0 a liquid composition comprising a test compound, a labeled ligand, and at least a ligand-interacting portion of a vanilloid receptor protein;
 - 10 (b) incubating the solution for a time sufficient to permit the test compound and labeled ligand to contact the vanilloid receptor;
 - (c) measuring the amount of labeled ligand bound to the protein; and
 - (d) determining if the test compound bound to the receptor by observing a reduction in the amount of expected labeled ligand.
 - 15 2. The method of claim 2 wherein the ligand-interacting portion of a vanilloid receptor protein is an intact vanilloid receptor protein.
 3. The method of claim 1 wherein the vanilloid receptor is a human vanilloid receptor.
 - 20 4. The method of claim 1 wherein the pH is in the range of about 8.0 to about 9.5.
 5. The method of claim 1 wherein the pH is in the range of about pH 8.1 to about 9.1.
 - 25 6. The method of claim 1 wherein the labeled ligand is a radiolabeled ligand.
 - 30 7. The method of claim 6 wherein the radiolabeled ligand is tritiated resiniferatoxin.

8. The method of claim 1 additionally comprising the steps after the incubating step of:

removing unbound labeled ligand from the solution; and
isolating the receptor protein;

9. The method of claim 1 wherein the aqueous buffer further comprises a divalent cation selected from the group consisting of:

- (a) magnesium at a final concentration of between about 1 to about 5mM; and
- (b) calcium at a final concentration of about 0.1mM to about 2 mM

10. The method of claim 9 wherein the magnesium concentration is about 2 mM.

11. The method of claim 9 wherein the calcium concentration is about 0.8 mM

12. The method of claim 9 wherein the vanilloid receptor is a human vanilloid receptor.

13. The method of claim 1 wherein the removing step comprises adding a sufficient quantity of alpha 1 acid glycoprotein to the aqueous solution to adsorb unbound labeled ligand.

14. The method of claim 1 wherein the steps are performed in order.

15. The method of claim 9 wherein the isolating step is performed before the removing step.

16. A method to measure ligand binding to a vanilloid receptor comprising the steps, in order

(a) combining in an aqueous solution having a pH in the range of about 7.5 to about 10.0, a test compound, a labeled ligand, and vanilloid receptor protein, said protein being associated with a portion of a cell membrane;

5 (b) incubating the solution for sufficient time for the test compound and ligand to contact the vanilloid receptor;

(c) adding a sufficient quantity of alpha1 acid glycoprotein to the solution to adsorb unbound labeled ligand;

(d) isolating the membrane from the aqueous solution;

10 (e) measuring the amount of labeled ligand bound to the protein in the membrane; and

(f) determining if the test compound bound to the receptor by observing a reduction in the amount of expected labeled ligand.

15 17. A method to measure compound binding to a vanilloid receptor comprising the steps, in order

(a) combining in an aqueous solution having a pH of about 8.6, a test compound, a radiolabeled resiniferatoxin, and a human vanilloid receptor-1 (VR1) protein, said protein being a portion of a cell membrane;

20 (b) incubating the solution for sufficient time for the test compound and the resiniferatoxin to contact the vanilloid receptor;

(c) adding a sufficient quantity of alpha1 acid glycoprotein to the solution to adsorb unbound resiniferatoxin;

25 (d) isolating the membrane from the aqueous solution;

(e) measuring the amount of resiniferatoxin bound to the protein in the membrane; and

(f) determining if the test compound bound to the receptor by observing a reduction in the amount of expected resiniferatoxin.

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18. The method of claim 17 wherein the buffer also contains a divalent cation selected from the group consisting of:

- (a) magnesium at a final concentration of about 2mM; and
- (b) calcium at a final concentration of about 0.8mM.

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